THE MEASURE OF HOOKWORM INFECTION IN COMMUNITIES

BY

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INTRODUCTION

In recent years the necessity for some measure of the degree, as well as the extent of hookworm infection in localities and communities, has been realised by a number of investigators, e.g., Darling (1922) and Cort (1924). It is an obvious but, nevertheless, largely ignored fact, that the percentage of individuals in whose stools eggs can be found is far from giving a true index to the severity of the hookworm situation, and yet, until very recently, this percentage has been accepted as the standard of measurement. The fallacy of this standard is, perhaps, nowhere more evident than in Bengal where, in spite of the fact that 70 per cent. or more of the population are infected, the individual infections are, on the whole, so light as to make hookworm disease in this province a comparatively unimportant problem. A correct estimate of the need for hookworm work and the judicious allocation of funds available for hookworm campaigns, as well as the strategy of campaigns, should depend on the degree as well as the prevalence of the disease. The relative value of different control measures and of different methods of treatment, also, can be correctly judged only by a consideration of the reduction in amount as well as in the incidence of infection. Hill (1923), for instance, showed that in certain areas in Porto Rico an intensive campaign reduced the percentage of infection from 87·2 to 34·1, but it reduced the egg output, which was used as a measure of the amount of infection, by 92·4 per cent.

METHODS OF MEASURING INTENSITY OF INFECTION

They may be classed as follows: (1) effects on host, (2) worm counts after anthelminthic treatment, and (3) estimation of the egg ouptut in the faeces.

Clinical symptoms, haemoglobin percentage and eosinophilia are the principal factors used in measuring effects on the host. All workers agree that the estimation of the amount of hookworm disease on the basis of clinical symptoms is difficult and complicated by differences in individual resistance, age, conditions of life, and concurrent disease; by the personal element in the classification of symptoms and severity of cases; and by the difficulty in making anything more than a very rough classification into light, moderate and severe cases. Such a clinical classification is of value in giving supplementary data as to the effects of the disease under local conditions, and in demonstrating individual and racial resistance, but it is of very little value, *per se*, as an indication of the degree of hookworm infection in a community. One might as well attempt to determine elevation on a mountain by reference to the permanent snow line, without consideration of other circumstances.

The haemoglobin content of the blood, as a measure of the degree of hookworm infection, is of little or no value in individual cases, although some authors, e.g., Darling, Barber and Hacker (1920), maintain that when sufficiently large numbers are averaged the amount of anaemia is proportional to the number of worms. Darling (1922) and Sawyer and Sweet (1922) have suggested definite ratios between the number of worms harboured and the percentage loss of haemoglobin. The haemoglobin content, however, is affected by so many factors such as sex, work, age, malnutrition, and such blood diseases as malaria, kala-azar, etc., that it can be used as a measure of hookworm infection only within wide limits. The process of elimination of other causes of anaemia is long and tedious, and in light cases there is usually no measurable drop in haemoglobin content. In a study of 100 individuals in the Alipore Central Jail, Calcutta, 67 of whom were infected with hookworm, but only six of whom had more than 1,000 eggs per gram of faeces, no difference in haemoglobin percentage between the infected and non-infected individuals could be found. The average for the uninfected ones,

according to the Tallquist scale, was 82·3, and for the infected ones 83. Two uninfected and only one infected case fell to 60 per cent., whereas one infected and one uninfected case reached 95 per cent. Darling (1922) shows that, in especially selected homogeneous groups, it requires fewer worms to cause a given loss of haemoglobin in a woman than in a man, and still fewer in a child, and also that a given number of *Ancylostoma duodenale* produces more anaemia than a similar number of *Necator americanus*, a fact which is now quite generally recognised. It is very probable that, as the number of worms increases, the haemoglobin content decreases at an accelerating rate, since it would become increasingly difficult for the patient to make good the loss produced by the worms.

Eosinophilia, as an indication of hookworm disease, is open to much the same criticisms as is the estimation of haemoglobin, since hookworm is only one of many causes of this condition. Practically all helminth infections produce more or less eosinophilia. Moreover, McVail (1922) observes that the eosinophilia in ankylostomiasis is not proportional to the number of worms present, even in uncomplicated cases, and he shows that kala-azar, and to a less extent malaria, is a powerful factor in reducing the eosinophilia due to helminthiasis.

The counting of worms passed after anthelmintic treatment, as a method of estimating the degree of infection in a community, is of unquestionable value when it can be properly carried out, but the difficulties involved are in most instances practically insurmountable. Only a small number of persons, and these especially selected ones, who can be relied upon to save all stools, can be examined in this way at a reasonable cost. The method requires a trained personnel, and cannot be left to subordinates; carelessness on the part of patients or laboratory staff, the partial failure of anthelmintics, and the loss of worms by maceration are all factors which interfere with the accuracy of the results. Even with every precaution which we have found practicable in the case of our hospital patients, we have not infrequently found lightly-infected cases to become microscopically cured after treatment, without finding any worms in the stools. It has usually been assumed that the washing of stools for 48 to 72 hours after treatment is sufficient to recover all of the worms passed, but even when a saline purge is given we have found

worms in stools as late as the sixth day after treatment, and quite frequently on the fourth day. Occasionally the stools have been negative on the second or third day, and again contained worms on the third or fourth day. For these reasons it is obvious that, however desirable a method for estimating degree of infection the worm count may be from a theoretical standpoint, it is certainly not in most instances practicable, and is always expensive.

The estimation of degree of infection by the egg output in the stools has very distinct advantages in the way of simplicity and practicability, providing that the egg output actually indicates the amount of infection. Even if this should prove to be true only to a limited extent and only when considerable numbers of individuals are averaged together, the knowledge of the number of eggs being deposited on soil, as Payne, Cort and Riley (1923) and Hill (1923) have pointed out, is, in itself, an important bit of information from the point of view of the spread of the disease. It is the egg output, and not the number of worms harboured or the clinical symptoms, which measures the public health menace.

ESTIMATION OF EGG OUTPUT

A number of different methods of estimating the actual or relative numbers of eggs in faeces have been utilised by different workers. Most of the methods of microscopic diagnosis can be used to give rough quantitative as well as qualitative information, but few of them are well adapted to give accurate information on this point. One of the first exact methods was Lane's (1918) ' standardising count,' which is Howard's centrifugal concentration technique reduced to accuracy of measurement, but this was not used for determining intensity of infection in groups or communities of people. The method devised by Stoll (1923a) is the only one which has been utilised in this way on a large scale. It is very simple, consisting merely of accurate dilution of a weighed sample of faeces in a decinormal NaOH solution, to clarify the fatty constituents of the faeces, and the accurate counting of a carefully-measured sample of the dilution. Lane (1924) has suggested the use of his direct centrifugal flotation method for this purpose, but, excellent as it may be for diagnosis, if the necessary apparatus is available,

it does not seem to me to be well adapted for quantitative work since, except where less than 500 eggs per gram are involved, the difficulty and tediousness of counting the great number of eggs thrown on the slide would counterbalance the advantage in reduced area of examination. It would be necessary, if numerous eggs were found, to repeat the process, using a much smaller quantity of stool, which would involve both time and inaccuracy due to the difficulty of measuring, say, 0·1 c.c. of stool.

The favourable results obtained by the use of Stoll's egg-counting method in Porto Rico led to a trial of it, with a few modifications, in Bengal. We have modified Stoll's method (1) by diluting the 3-gram sample of faeces to 90 c.c. instead of 45 c.c. ; (2) by counting the eggs in a 0·3 c.c. sample of the dilution instead of 0·15 c.c. ; and (3) by examining the preparation uncovered. There are several advantages in these modifications. In searching for eggs on an area of about two square inches on an uncovered slide, marked off by means of a glass pencil, it was found that 0·3 c.c. of the faecal suspension was necessary, under tropical conditions, to prevent the preparation from partial drying before the examination was complete. In order to get a sufficiently clear field for examination of ordinary stools with this quantity of the suspension, a dilution of 1 to 30 instead of 1 to 15 was necessary. The greatest advantage of using the larger amount of fluid and examining the preparation uncovered lay in the ability lightly to blow aside the flocculent masses of débris which often tend to hide the eggs, by gently puffing on the slide while the examination is actually in progress. Camouflage of eggs by débris is the most important source of error in all the techniques in which accurate measurements of material are made. Lane (1923, 1924) gives convincing evidence of the loss of eggs by camouflage. Maplestone (1924), in testing Stoll's method, nearly always obtained higher counts per gram when the faeces were further diluted before the egg count was made, obviously due to overlooking of eggs as the result of concealment in the more concentrated samples. By using a suspension in decinormal NaOH on an open slide with 0·3 c.c. of fluid spread over an area of 2 square inches, it is ordinarily possible, by gently blowing on the slide while making the examination, to see practically 100 per cent. of the surface of the slide with sufficient clearness to render the eggs easily visible. The eggs are heavier than

the flocculent material which makes up the great bulk of the débris on the slide, and, therefore, rest on the slide and remain visible as the overlying material is puffed aside. Furthermore, one can almost instantaneously determine whether or not an object which resembles an egg is such, since its position can be slightly changed or it can be rolled over by the same gentle puffing process. In very concentrated formed stools, we sometimes find it necessary to divide the 0·3 c.c. sample on two slides and dilute them further.

There are a few possible sources of error in this method which may be briefly commented on. In the first place, the selection of a 3-gram sample of faeces should, when possible, be made from an entire stirred stool, since the number of eggs contained in different parts is not always the same. Making duplicate counts on two samples from different parts of a single stool, the widest differences we obtained were counts of 700 and 900 eggs per gram on one sample and 1,600 and 1,800 on the other. After stirring this stool, examination of a third sample gave two counts of 1,200. In field work it is usually not practicable to get entire stools, and counts must be made on the samples submitted. As will be subsequently shown, however, the error arising from this, in individual cases, is neutralised when 50 or 100 samples are averaged.

We have tested a number of diluting fluids but found that the decinormal NaOH solution gave clear fields and more readily visible eggs than any other fluid. Addition to the NaOH of 1·5 per cent. NaCl had the effect of causing the faecal débris to clump together into large light flocculent masses which could be blown about, leaving a beautifully clear background on which the eggs showed up with striking clearness, but the occasional entanglement of eggs in these masses reduced its accuracy.

The thorough mixing of the samples in homogeneous suspensions is sometimes slow and difficult, and it is easy to overlook small masses of faeces which have failed to disintegrate. Unless carefully watched, this is one of the most fruitful sources of error. When available, a mechanical shaker is of great advantage. Settling of eggs in the diluting fluid must also be carefully guarded against ; the stopper of the flask should be removed and the sample withdrawn immediately after a thorough and vigorous shaking. Even a few seconds' delay entails inaccuracy. We have found that the samples

can be withdrawn more quickly and accurately into rubber-bulb pipettes of drawn glass tubing marked at the 0·3 c.c. level, than into bacteriological pipettes. Only the required amount of fluid is sucked into the pipette, and all of it expelled on to the slide.

The NaOH solution does not appreciably change the appearance of the eggs of hookworms, *Trichuris*, *Hymenolepis nana*, or *H. diminuta*, but Ascaris eggs have the rough albuminous coat more or less completely dissolved off and thus often look quite different from the normal eggs, especially in the case of unfertilised ones. Taenia eggs undergo a peculiar change in that the embryophore swells to a diameter of from 50 to 60μ, leaving a much-enlarged clear space between it and the embryo ; the latter shrinks somewhat and assumes a characteristic elongated form.

Recounts of the same slide, duplicate counts from the same suspension, and counts on higher dilutions have shown that camouflage of eggs is practically done away with by the method here described. Lane warns against the loss of eggs held on the surface of a film too deep to be in one optical plane and in which only the bottom is searched. Apparently this rarely happens in a decinormal NaOH solution since I have several times gone over the surface of a slide containing several hundred eggs without finding a single egg. The entanglement of eggs in flocculent masses occasionally occurs, though much more frequently with Ascaris than with hookworm eggs. It usually takes a little time for the débris on the slide to clump, a process which takes place much more extensively in some stools than in others ; consequently, it only rarely happens that the eggs do not have time to settle. The blowing process also aids in liberating them. There is no doubt but that some loss of eggs does occur in these ways, but even if there were a constant loss of, say, 10 per cent. of eggs, it would be of little consequence, since what is desired is not so much an absolute knowledge of the number of eggs as a comparative measurement of the egg output.

That the method here described gives a good comparative measurement of eggs per gram of faeces is shown by the uniformity of counts which are obtained from examinations of two different samples prepared from the same stool. Where the average count on the slide is 10 or less, in about 80 per cent. of several hundred duplicate examinations, the counts were identical or within one

of each other, and, therefore, as close as possible to the average. In another 16 per cent. the counts were two numbers apart, whereas in only about 4 per cent. were the counts three numbers apart. Where the average slide count is between 10 and 100, in 35 per cent. the two counts came as near as possible to the average, in another 44 per cent. they were not over 10 per cent. from the average, whereas in only 8 per cent. were they more than 15 per cent. from the average. Where the average count exceeded 100, 87 per cent. of the duplicate counts fell within 10 per cent. of the average and none over 15 per cent. from it.

In nearly every instance in which there was any considerable discrepancy in the two counts a clumping of the eggs was observed, evidently due to their being held together by strands of mucus which had not been broken up in the shaking, in spite of an apparently homogeneous suspension. This clumping was also observed by Davis (1924), but with our technique we have only rarely obtained as irregular duplicate counts as Davis records in many of his cases; undoubtedly he was dealing with mucous stools.

In a series of about 600 faecal samples received from the Alipore Central Jail, counts have been made on two different slides prepared from a single suspension made from samples collected in quarter-ounce faeces-tins. The results which have been obtained from these counts compare very closely with those obtained from examinations of two separately prepared suspensions. This indicates that the differences in the counts are due not to variations in different parts of a stirred stool, but to errors in the counting technique. It appears, therefore, that a single suspension made from a stirred stool gives a sufficiently fair sample of the entire stool.

RELIABILITY OF EGG COUNTS AS AN INDICATION OF DEGREE OF INFECTION

It is now important to know the amount of variation which occurs in the eggs per gram of faeces in individuals according to the consistency of the stool, and from day to day. To get some light on this we studied the egg content of the stools of 36 hospital patients on from 3 to 22 different days, and made duplicate egg counts on separately prepared suspensions from 194 stools. By classifying the

stools as liquid, mushy, semi-formed and formed, and comparing the egg counts of these several groups in the case of each individual, it soon became apparent that, roughly speaking, the formed stools contained twice as many eggs and the liquid stools half as many or less, as the mushy stools. This compares fairly closely with Stoll's findings in Porto Rico (1923b). It was evident, therefore, that if intensity of infection were to be measured by egg counts, the factor of consistency would have to be considered.

Since, in India, mushy stools are normal and formed ones are rare, we accept the count on mushy ones as normal and correct the counts on formed and liquid stools by dividing or multiplying by 2. Such counts we refer to as 'corrected counts.' In a paper which has recently come to hand, Stoll (1924) arrives at exactly similar conclusions, except that he accepts formed stools as normal and multiplies the counts on mushy and liquid stools by 2 and 4 respectively, to bring them to 'basis of formed stool.'

Our counts on these preliminary 36 patients showed, however, that even when the consistency of the stool does not vary, there is a surprising variation in the egg output per gram of faeces on different days. In case 22, for instance, considering only the mushy stools, there was a maximum variation from 250 to 1,100 eggs per gram, in case 29 from 500 to 1,250, in case 32 from 250 to 1,000, and in case 35 from 50 to 350. These are the most extreme cases; in most instances, if the consistency of the stool is taken into consideration, the variation is much less. There appears to be a much more marked tendency to vary in some individuals than in others. Stoll (1924), in a study of the egg output of two individuals, for 15 and 40 days respectively, found a similar day-to-day variation. In one of his cases the mushy stools varied from 1,000 to 2,600 and in another from 430 to 800, whereas the formed stools in the latter case varied from 400 to 1,330.

An attempt was made to get 24-hour samples of stools and to calculate the total daily egg output for 24 hours by means of the egg count and stool weight, since it seemed probable that the amount of the stool would to some extent counterbalance the variations in eggs per gram. Our results, however, failed to show any such counterbalancing tendency, since it just as frequently happened that a low egg count was accompanied by a small 24-hour output of stool

as the reverse, thus giving a greater variation in the total egg output than had been found in the number of eggs per gram. Stoll's (1924) tables show a similar lack of correlation. The most obvious reason for this appears to be that the extent to which the bowels are emptied on each day varies, even if the habits are fairly regular. In most of my cases the hour at which the stools are passed each day varies considerably, so it occurred to me that better results might be obtained by weighing only a single stool each day and keeping a record of the time between the last previous stool and the one examined. In this way we should know the number of hours during which the faeces and the eggs contained in them had been accumulating and could calculate from this the number of eggs produced in 24 hours. 112 stools from 23 different cases were examined in this way, but practically the same amount of variation was found in daily output as when 24-hour outputs of faeces were weighed without reference to time of stools, undoubtedly due to the same factor of completeness of evacuation of the bowels.

As Stoll has pointed out, it is only when the total daily output of eggs, calculated from eggs per gram and weight of stool, is averaged for at least three days that the coefficient of variation is reduced to a low level. For one-day examinations the egg count by itself gives less variable results than the calculated total egg output. Since, under field conditions, the collection and weighing of stools for three days on any considerable number of individuals is out of the question, for the same reasons that worm counts are impracticable, reliance must be placed on the egg counts alone, even though some inaccuracy is involved. Stoll has shown that in the two cases he examined, which were of widely different types, the total egg outputs showed a relation of 5·4 : 1, whereas the average corrected egg counts per gram showed a relation of 3·3 : 1. The failure of the egg counts to show a correct relationship is, of course, due to differences in food habits and consequent daily amount of faeces in which the eggs are distributed. We believe, however, that in more or less homogeneous groups, such as tea garden coolies, mine labourers, etc., habits are sufficiently alike for the corrected egg count, if averaged for three days, to give a reasonably good index of the relative egg output of different individuals. Since the egg counts of individuals approach a level when averaged for three or four days, it is obvious that in

determining the degree of infection of a group or community by counts on 50 or 100 individuals, single egg counts are quite sufficient, since variations would automatically be blotted out in the consideration of such numbers.

To test this point a study was made of 100 prisoners in the Alipore Central Jail, with the kind co-operation of the Superintendent, Lt. H. A. Young, I.M.D. A double count was made from a single suspension on two separate occasions, about a week apart. Most of the infections found were extremely light, so that although 67 were shown to be positive for hookworm, by the Kofoid and Barber technique, only 45 positives were found by examination of two slides prepared for egg counts on the first examination, and 44 on the second. Eleven which were negative on the first examination were positive on the second, and 12 which were positive on the first were negative on the second. Of these 23 cases, 12 showed only a single egg on four slides, six more gave an average of one egg per slide on the positive examination, and the remaining five gave average counts of from 1·5 to 2·5 on the positive examination. In spite of the high percentage of these low counts, which would tend to increase the probable error in the two counts, the average number of eggs per gram of faeces on the first examination was 282 and on the second 257, a deviation of only 4·6 per cent. above and below the average of the two. This compares quite favourably with the deviations of 3·9 per cent. and 3·3 per cent. from the average, which were found in the first and second slides in the first and second examinations respectively. This justifies the conclusion that a single egg count on a fair number of individuals gives a reasonably good estimate of the average egg output of that group.

Owing to the fact that we have not found it practicable to control our hospital patients sufficiently so that the preservation of all stools passed after anthelmintic treatment could be depended upon, we can give no reliable statistics on the relationship between egg counts and worms harboured. In cases in which we have reason to believe that all the stools were saved, the number of eggs per gram per female worm usually falls between 8 and 20. In one instance, however, in which duplicate counts were made for three successive days, without finding any eggs at all, although the case was positive by flotation, four female Necators were passed. In another case which

passed 16 female Necators two eggs were found on each of two slides on one day and no eggs on duplicate examinations on two subsequent days; in this case something must have inhibited oviposition on these two days. There is likely to be less variation of this kind in the field than in a hospital, where alterations in diet, drug treatments, and concurrent disease may influence both the quantity of the stool and the oviposition of the worms.

That the correlation between egg count and worms harboured is not close in individual cases is evident from the day to day variations in the count. Mhaskar (1923) gives a table of 30 cases which purports to show that there is no correlation at all. Darling (1922) on the other hand, gives a table in which a distinct correlation is shown. Smillie (1921) and Stoll (1923b) also find a correlation. On purely theoretical grounds one is forced to the conclusion that, other things being equal, there *must* be some relationship between egg output and number of worms harboured. For instance, if a patient harbouring 10 female worms produced, on successive days, 100, 500, and 200 eggs per gram of faeces, is there any reason to doubt that if he harboured 20 female worms, other conditions being the same, he would pass on each of these days approximately twice as many hookworm eggs? It is reasonable to assume, then, that when the egg output of a large number of representative individuals is averaged together, this number gives a sufficiently accurate estimate of the degree of infection so that it can be used for comparison of different groups of individuals living under similar conditions and having similar food habits, or of the same groups before and after treatment, or for the establishment of control measures. The average eggs per gram is a less accurate guide in comparing groups living under quite different conditions and having widely different food habits, but even here, within wider limits, rough comparisons can be made. This is, however, of far less value and importance, for practical purposes, than the comparison of different groups of a single area by age, sex, occupation, etc., and the comparison of such groups at different times for the valuation of the effectiveness of control measures.

ESTIMATION OF INFECTION INDEX

Although Cort (1924) suggests the substitution, in surveys, of the egg counting method for the routine faecal examinations now generally used, and describes hypothetical cases which show its advantage, it seems to me that there is fallacy in accepting either the degree of infection as determined by worm or egg counts, or the mere percentage of incidence of infection, as an index of the amount of hookworm infection in a community, or of the benefits derived from treatment or control measures. For example, let us suppose that in two communities both living under climatic and soil conditions favourable for the propagation of hookworm, the number of eggs per gram of faeces averages exactly the same, but that the sanitary conditions and habits of the people differ. In one community the majority of the people are sanitary in habits and the hookworm infection is largely confined to a few families who are backward and careless in habits, while in the other community sanitary conditions throughout are not so good and the infection is more uniformly scattered through a high percentage of the people. In such a case it is clear that the two groups should not be placed on a par, as would be the case if only the degree of infection for the group, based on egg output, were considered ; nor should the condition of the first community be considered as far superior to that of the second as the difference in percentage of infection would probably place it. From the standpoint of the general effect on the community, the probable spread of the disease, and the sanitary conditions indicated, it is important to take into consideration the number of individuals among whom the egg output is divided. Certainly the higher the percentage of individuals who are scattering a given number of hookworm eggs daily, the greater the opportunity for the spread of the disease, and the more important it is that control measures should be inaugurated. One hundred individuals each with an output of 100 eggs per gram of faeces certainly constitute a greater menace to the community than ten individuals each with an output of 1,000 eggs per gram, or one individual with an output of 10,000 per gram, since, although the total number of eggs produced is the same in each instance, the extent to which they are scattered is largely proportional to the number of persons who are passing them, and the more they are

scattered the more opportunity there is likely to be for the larvae which develop from them to gain access to new hosts. The incidence of infection, then, rather than the degree of infection, is the correct measure of the extent to which the entire community has been, and is likely to be, exposed to the infection, whereas the degree of infection rather than the incidence of it is a rough measure of the extent to which individuals have been, and are likely to be, exposed, and of the facility with which infection can occur, under the climatic and soil conditions of the locality, when carelessness in habits permits it.

It seems to me, therefore, that both factors must be taken into consideration in order to arrive at a true hookworm infection index. To do this I have tried various ways of combining the incidence and degree of infection, as indicated by eggs per gram of faeces, to obtain a number which would give a true relative index in various actual and hypothetical cases, as judged by a common-sense consideration of all the facts involved Such an index number can, I think, be obtained by taking the square root of the product of the average eggs per gram, multiplied by the percentage of infection, or, alternatively, by taking the square root of the product of the egg counts, averaged for the infected individuals only, multiplied by the square of the percentage infected, i.e., by the equation :

$\sqrt{\frac{e.p.g.}{100} \times \%^2} = I$, where *e.p.g.* stands for average eggs per gram of the infected individuals ($\frac{e.p.g.}{100}$ being the average of the eggs counted on the slides), % the percentage infected, and *I* the resulting infection index. For example, if 50 of 100 individuals have an average of 400 eggs per gram by corrected counts, the other 50 having none, the equation would be : $\sqrt{\frac{400}{100} \times 50^2} = 100$, which is the infection index. The three hypothetical cases mentioned above of a 100 per cent. infection with 100 eggs per gram, a 10 per cent. infection with 1,000 eggs per gram, and a 1 per cent. infection with 10,000 eggs per gram, are all on a par on the basis of degree of infection for the group ; they stand in the ratio of 100 : 10 : 1 on the basis of incidence of infection ; while their infection indices work out at about 100 : 32 : 10, which seems to come much nearer their true relationships. It will be seen, however, that this method of calculation

gives correct results only if all the infections are uniform, since the average implies that the egg output is evenly divided among all the infected individuals, which is seldom the case. To get a correct estimate, therefore, the entire group of infected individuals should be broken arbitrarily into sub-groups according to the size of the egg counts, and the infection index for each sub-group separately figured and then all of them added together. For example, in a community with a 60 per cent. infection, 20 per cent. with egg counts of 100 to 500 (average 300), 20 per cent. with counts of 500 to 2,100 (average 1,000) and 20 per cent. with 2,100 to 5,100 (average 3,000), the infection index, if figured for the entire group, would be: $\sqrt{\frac{1433}{100} \times 60^2} = 228$, whereas if figured for each group separately, the infection index works out as follows: $\sqrt{\frac{300}{100} \times 20^2} + \sqrt{\frac{1000}{100} \times 20^2} + \sqrt{\frac{3000}{100} \times 20^2} = 209$. We consider as very satisfactory the grouping used by Payne, Cort and Riley (1923), according to the following numbers of eggs per gram: 1-599, 600-2,099, 2,100-5,099, 5,100-11,099, and 11,100 up.

Table I gives the infection index, as worked out on a number of actual cases, based on my own work in Bengal and on statistics given by Payne, Cort and Riley (1923), and Hill (1923), in Porto Rico. It should be noted, however, that the Jute Mill statistics are not entirely correct, since the entire percentage of infection was not determined by a concentrative method, and therefore, as the egg counts run very low, a considerable number of light infections would probably be passed over by the egg counting technique, as was shown by the Alipore Jail investigation mentioned above. It is necessary, therefore, that the egg-counting method be supplemented by a concentrative technique in order to discover the light infections which would otherwise be missed. In calculating the infection index those cases which are positive by the concentrative method only, and negative on two egg-count slides, can be calculated arbitrarily as having 25 eggs per gram.

The method we have adopted, therefore, as a routine for determining the infection index, and which we recommend for general use, is as follows :—

TABLE I.

	Number Examined	% with 1-599 e.p.g.	Average e.p.g.	% with 600-2099 e.p.g.	Average e.p.g.	% with 2100-5099 e.p.g.	Average e.p.g.	% with 5100-11099 e.p.g.	Average e.p.g.	% with 11100 or more e.p.g.	Average e.p.g.	Total % infected	Average e.p.g. for the entire group	Index of Infection
Coolies in Jute Mills and Coolie Lines	143	36†	260	18	1020	4	3100	1	5700	...	...	58†	420†	140†
Coolies in Jute Mills and Coolie Lines	48	19†	275	17	1160	2	3400	...	...	...	...	38†	310†	100†
Coolies living outside Jute Mill	98	44†	242	14	830	...	...	...	...	...	...	58†	220†	109†
Prisoners in Allipore Jail (1st exam.)	100	56**	133	9	810	1	3200	1	8500	...	...	67	272	106
Prisoners in Allipore Jail (7 days later)	100	57**	144	7	1040	2	3320	1	6450	...	...	67	286	110
Cases in Porto Rico, Area C (Payne, Cort and Riley)	92	17	300*	16	1350*	25	3600*	20	8100*	17	15000*	96	7740	630
Cases in Porto Rico Areas (before treatment) (Hill)	282	28	300*	27	1350*	17	3600*	9	8100*	6	15000*	88	2820	408
Cases in Porto Rico Areas (after treatment) (Hill)	282	25	300*	6	1350*	3	3600*	1	8100*	·35	15000*	35	215	92

* These are means instead of averages, data for the latter not being available.
† These figures are too low, since no concentration method was used to discover infections too light for detection by the egg-counting method.
** These include 23 cases detected by concentration method, but negative by egg-counting method. These infections were arbitrarily figured as having 25 e.p.g.
The figures in all cases are given to the nearest integer.

(1) Determination of the incidence of infection by a concentrative technique. In my experience the Kofoid and Barber method has given the most uniformly satisfactory results ; according to tests we have made it is more accurate than the Willis method or any of the usual centrifuge methods. If the necessary equipment is at hand the published evidence in favour of Lane's direct centrifugal flotation method indicates it as the method of choice, but since our centrifuges are not adapted to this method I have not had an opportunity of trying it myself. Neither the Kofoid and Barber, nor the Willis methods are reliable for light Ascaris infections ; to detect these we have found Lane's levitation method the most satisfactory.

(2) Determination of eggs per gram, by examination of all positives, by the modification of the Stoll egg-counting technique here described. By preference two slides should be examined and averaged, and the count corrected according to the consistency of the stool ; if, however, 50 or 100 specimens are examined, the total averages, though not the individual counts, will be very nearly correct if only one slide is examined of specimens showing two or more eggs. Specimens found positive by the concentrative technique, but negative on two egg-count slides, may be arbitrarily calculated as having 25 eggs per gram.

(3) Determination of the infection index by the equation : $\sqrt{\frac{e.p.g.}{100} \times \%^2}$ = Infection Index, where *e.p.g.* stands for the average eggs per gram of the infected individuals, and where the equation is separately figured for different groups showing different degrees of infection, as suggested above.

In conclusion I take pleasure in acknowledging the painstaking and reliable help given by my assistant, Dr. A. K Mukerji.

SUMMARY

1. Recent investigations have shown the necessity for the measurement of the degree of hookworm infection as well as its incidence ; such a measurement is of value from the point of view of the urgency, nature and valuation of methods of treatment and control.

2. Degree of infection may be measured by clinical symptoms, haemoglobin content, eosinophilia, worm counts after treatment, or by estimation of egg output in the faeces. The first three are not reliable, and the worm counts are too difficult, impracticable and expensive for use on a large scale.

3. Estimation of egg output is simple and practical, and is of value in itself as an accurate measure of potential soil pollution whether or not it indicates accurately the number of worms harboured. A modification of Stoll's egg-counting method is described and recommended for general use. Uniformity of duplicate counts indicates that it gives a good comparative measurement of egg output.

4. The consistency of the stool must be taken into consideration in estimating the egg output from the number of eggs per gram of faeces. Counts on formed or liquid stools, where mushy stools are normal, as in India, can be corrected by dividing or multiplying by 2. Day-to-day variations in eggs per gram are considerable, but the counts approach a level when averaged for three or more days. Consideration of the quantity of the stool does not lessen the variation unless averaged for at least three days, and is, therefore, not practicable in field work. The corrected egg count alone must be relied upon, and in more or less homogeneous groups we believe that this gives a reasonably good indication of the relative degree of infection in different individuals. When averages of large groups are being considered, single egg counts of individuals are sufficient.

5. In individual cases the correlation between egg counts and number of worms harboured is not very close, but when the egg counts for a group are averaged, a fair estimate of the relative numbers of worms harboured can be obtained, especially when homogeneous groups, or the same groups at different times, are compared.

6. It is not advisable to measure hookworm infection in a community by the degree of infection alone ; the incidence should also be considered, since the higher the percentage of individuals who are scattering a given number of eggs, the greater the danger to the community. The incidence of infection is a measure of the extent to which the entire community is exposed to infection ; in a general way it measures sanitary conditions. The degree of

infection, on the other hand, is a measure of the facility with which infection can occur under the climatic and soil conditions of the region when carelessness in habits permits it.

7. A good infection index can be obtained only by taking both factors into consideration. This can be done by means of the equation : $\sqrt{\frac{e.p.g.}{100}} \times \%^2 =$ Infection Index, where *e.p.g.* stands for average eggs per gram of infected individuals only. This equation should be separately figured for different groups falling into certain abritrary divisions according to number of eggs per gram, and all of them added together.

8. It is recommended that the infection index in survey work be determined as follows : (1) determination of incidence of infection by a concentrative technique ; (2) estimation of the degree of infection by means of egg-counts ; and (3) determination of the index of infection by the equation given above.

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